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Chronic Airway Colonization by *Achromobacter xylosoxidans* in Cystic Fibrosis Patients Is Not Sustained by Their Domestic Environment

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ABSTRACT *Achromobacter* spp. are nonfermentative Gram-negative bacilli considered emergent pathogens in cystic fibrosis (CF). Although some cross-transmission events between CF patients have been described, *Achromobacter* strains were mostly patient specific, suggesting sporadic acquisitions from nonhuman reservoirs. However, sources of these emergent CF pathogens remain unknown. A large collection of specimens ($n = 273$) was sampled in the homes of 3 CF patients chronically colonized by *Achromobacter xylosoxidans* with the aim of evaluating the potential role of domestic reservoirs in sustaining airway colonization of the patients. Samples were screened for the presence of *Achromobacter* by using genus-specific molecular detection. Species identification, multilocus genotypes, and antimicrobial susceptibility patterns observed for environmental isolates were compared with those of clinical strains. Patient homes hosted a high diversity of *Achromobacter* species ($n = 7$), including *Achromobacter mucicolens* and *A. animicus*, two species previously isolated from human samples only, and genotypes ($n = 15$), all showing an overall susceptibility to antimicrobial agents. *Achromobacter* strains were mostly isolated from indoor moist environments and siphons, which are potential reservoirs for several CF emerging pathogens. *A. xylosoxidans*, the worldwide prevalent species colonizing CF patients, was not the major *Achromobacter* species inhabiting domestic environments. *A. xylosoxidans* genotypes chronically colonizing the patients were not detected in their household environments. These results support the notions that the domestic environment could not be incriminated in sustained patient colonization and that after initial colonization, the environmental survival of *A. xylosoxidans* clones adapted to the CF airways is probably impaired.

IMPORTANCE *Achromobacter* spp. are worldwide emerging opportunistic pathogens in CF patients, able to chronically colonize the respiratory tract. Apart from regular consultations at the hospital CF center, patients spend most of their time at home. Colonization from nonhuman sources has been suggested, but the presence of *Achromobacter* spp. in CF patients' homes has not been explored. The domestic environments of CF patients chronically colonized by *Achromobacter*, especially wet environments, host several opportunistic pathogens, including a large diversity of *Achromobacter* species and genotypes. However, *Achromobacter* genotypes colonizing the patients were not detected in their domestic environments, making it unlikely that a shuttle between environment and CF airways is involved in persisting colonization. This also suggests that once the bacteria have adapted to the respiratory tract, their survival in the domestic environment is presumably impaired. Nevertheless, measures for reducing domestic patient exposure should be targeted on

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evacuation drains, which are frequently contaminated by CF opportunistic pathogens.

KEYWORDS *Achromobacter*, domestic environment, cystic fibrosis, habitat, drains, reservoir, chronic colonization, diversity, antimicrobial resistance

Achromobacter is an opportunistic environmental bacterium identified in soil and water samples. This genus is nowadays considered a worldwide emerging pathogen in cystic fibrosis (CF) (1–3), with *Achromobacter xylosoxidans* being the most prevalent species (1, 2, 4). Except for outbreaks described in some CF centers (5, 6), studies investigating the diversity of *Achromobacter* spp. isolated from the airways of CF patients showed that *Achromobacter* spp. mostly originate from sporadic acquisition rather than from cross-transmissions or from the diffusion of a limited number of clones with epidemic success (1, 7, 8). While CF patients regularly visit hospitals for routine visits and to receive intravenous antibiotics, they spend much more time at home than at the hospital. A few studies investigated sources of pathogen acquisition by CF patients outside the hospital but were mainly focused on *Pseudomonas aeruginosa*. *P. aeruginosa* clone C, which is epidemic in CF, was isolated from different habitats, including river, pool or tap water, suggesting a genotype with a versatile lifestyle and diversified potential sources for acquisition (9). In domestic environments, even fewer investigations were conducted in both CF and non-CF patient homes and showed *P. aeruginosa* to be mainly localized in drains used for carrying off surplus liquid waste (10–12). However, in these studies, *P. aeruginosa* genotypes have not been studied, impairing any comparison between environmental and human strains. An investigation conducted in 50 homes of CF patients as soon as possible after the diagnosis of a first *P. aeruginosa* infection found (i) a low prevalence of *P. aeruginosa* in the home environment (5.9% of the samples, 18 patient homes) and (ii) identical genotypes of *P. aeruginosa* determined by amplified fragment length polymorphism in the domestic environment, mainly in bathroom samples, and in the patient airways in 9 cases (18% of the patients) (13).

Regarding *Achromobacter* spp., two environmental studies dedicated to searching for *A. xylosoxidans* were conducted on environmental samples of various origins but not in CF patient households. In those studies, *Achromobacter* appeared to be widely distributed in natural hydrosystems, houses, and hospitals (14, 15). In the present study, we assessed distribution and diversity of *Achromobacter* spp. in the homes of 3 CF patients chronically colonized by *A. xylosoxidans* with the aim of evaluating the potential role of home reservoirs in sustaining airway colonization of the patients.

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RESULTS AND DISCUSSION

***Achromobacter*-specific PCR *in silico* performances.** Based on the *Proteobacteria* 16S rRNA gene aligned sequences ($n = 4{,}592$), only three regions allowing primer design (20 to 30 bp) showed sequences conserved among members of the *Achromobacter* genus and distinct from those of other genera. The two most distant sequences were chosen to design forward primer 638-F (5′-CGCAGGCGGTTCGGAAAGAAA-3′) and reverse primer 810-R (5′-GCCTCCTGGGATAACACTGA-3′), allowing the amplification of a 174-bp product. Both primers exactly anneal to the sequences for type strains of 16 *Achromobacter* species available in RDP-II but also to sequences for type strains of 8 *Bordetella* species. If 2 nucleotide mismatches per primer are allowed, 9 other genera were detected: *Advenella*, *Alcaligenes*, *Kerstersia*, *Pelistega*, *Pigmentiphaga*, *Pusillimonas*, *Taylorella*, *Candidimonas*, and *Eoetvoesia*. Using the primer targeted on the third consensual region (amplification product of 120 bp) gave no gain in specificity (data not shown).

***Achromobacter* detection by specific PCR and cultivation on selective medium.** The performance of the *Achromobacter* selective agar medium described by Amoureux

TABLE 1 Description and obtained results for *Achromobacter* sp. isolates recovered from domestic environment samples[a]

Sample ID	Room	Isolation source	*Achromobacter* species	ST/MLST	Associated bacteria identified by MALDI-TOF
P2-20	Bathroom	Right sink siphon water	*A. aegrifaciens*	350	*A. radiobacter*, *P. nitroreducens*
			A. aegrifaciens	350	
P2-97	Garden	Inside water hose swab	*A. animicus*	360	*Bordetella* sp., *Pseudomonas* sp.
P2-101		Plant (+roots)	*A. spanius*	352	
			Achromobacter gen. 9	351	
P2-106		Flowerpot soil	*A. mucicolens*	353	*A. radiobacter*
P5-11	Kitchen	Sink siphon water	*A. marplatensis*	354	*Ochrobactrum* sp.
P5-56	Bathroom 1	Washing machine evacuation	*A. mucicolens*	355	*Bordetella* sp., *S. maltophilia*, *Ochrobactrum* sp.
P5-58		Bath mat	*A. spanius*	356	
P5-50	Bathroom 2	1st-flush sink water	*A. xylosoxidans*	175*	*A. radiobacter*
P5-64	Other	Tumble dryer reservoir	*A. xylosoxidans*	175*	
P5-71		Floor cloth bucket water	*A. xylosoxidans*	175*	*O. anthropi*, *O. grignonense*, *Ochrobactrum* sp.
			A. xylosoxidans	169*	
P5-72		Floor cloth	*A. xylosoxidans*	175*	*Ochrobactrum* sp.
P5-74	Garden	Vegetable garden soil	*A. mucicolens*	357	
			A. mucicolens	358	
P12-26	Kitchen	Washing machine evacuation	*A. aegrifaciens*	143*	*Bordetella* sp., *O. anthropi*, *A. radiobacter*
P12-49	Bathroom	Shower siphon water	*A. mucicolens*	359	*P. nitroreducens*, *Pseudomonas* sp., *Ochrobactrum* sp.
P12-61		Toothbrush glass	*A. mucicolens*	349	*A. radiobacter*
			A. mucicolens	349	
			A. mucicolens	349	

[a]Sample identification (ID) is composed of the patient number followed by the sample number. *, ST already described in PubMLST database. gen., genogroup; A., *Agrobacterium/Rhizobium*; P., *Pseudomonas*; S., *Stenotrophomonas*; O., *Ochrobactrum*.

et al. has not been fully validated, i.e., evaluated for all species in the genus *Achromobacter* or a variety of strains within a species (15). Therefore, to overcome a potential culture bias, the strategy performed in this study was to screen cultures obtained on a nonselective medium by applying the genus-specific PCR before cultivating the samples onto the *Achromobacter* selective agar medium. Among the 273 cultivable communities obtained on Trypticase soya (TS) agar, 92 showed a positive *Achromobacter*-specific PCR result, indicating that the prevalence of members of the genus *Achromobacter* was 33.7% in the collection of 273 samples from CF patient houses.

From the 92 corresponding cultures on the *Achromobacter* selective medium, a total of 316 colonies corresponding to different morphotypes were identified by matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) and 21 isolates from 15 samples were affiliated with the genus *Achromobacter* (13 with an identification score between 1.70 and 1.99 and 8 strains with a score of $\geq$2 using the Microflex Biotyper database [Bruker, USA]). In 11 of these 15 samples, *Achromobacter* was cocultured on the selective medium with members of other bacterial genera, including *Bordetella* (Table 1). *Achromobacter* species strains were isolated by culture from only 16.5% of the PCR-positive samples.

The low rate of isolation is possibly due to the partial selectivity of the medium, allowing the growth of some other genera than *Achromobacter* that may hide the growth of *Achromobacter* in the case that the inoculum is of low density. Moreover, the growing capacity of the selective medium was not evaluated for all of the 19 species currently described for the *Achromobacter* genus (15). False-positive PCR by cross-reaction with 10 predicted genera is unlikely to explain the observed level of discrepancy between PCR and culture. Particularly, *Bordetella* was isolated from only 10% of PCR-positive samples and coisolated with *Achromobacter* strains in only 3 samples.

As generally observed for targeted approaches of bacterial diversity, the full recovery of all the members of the genus was not warranted and the diversity of *Achromobacter* spp. may be higher than described. Metabarcoding that avoids cultural bias can be proposed for accurate detection of *Achromobacter* in domestic environments as previously performed for indoor community description (16). However, coupling detection by specific PCR and cultivation on selective medium as described here provide a useful tool for studies aiming for prevalence assessment in the environment while also recovering living material for further genotyping and phenotyping.

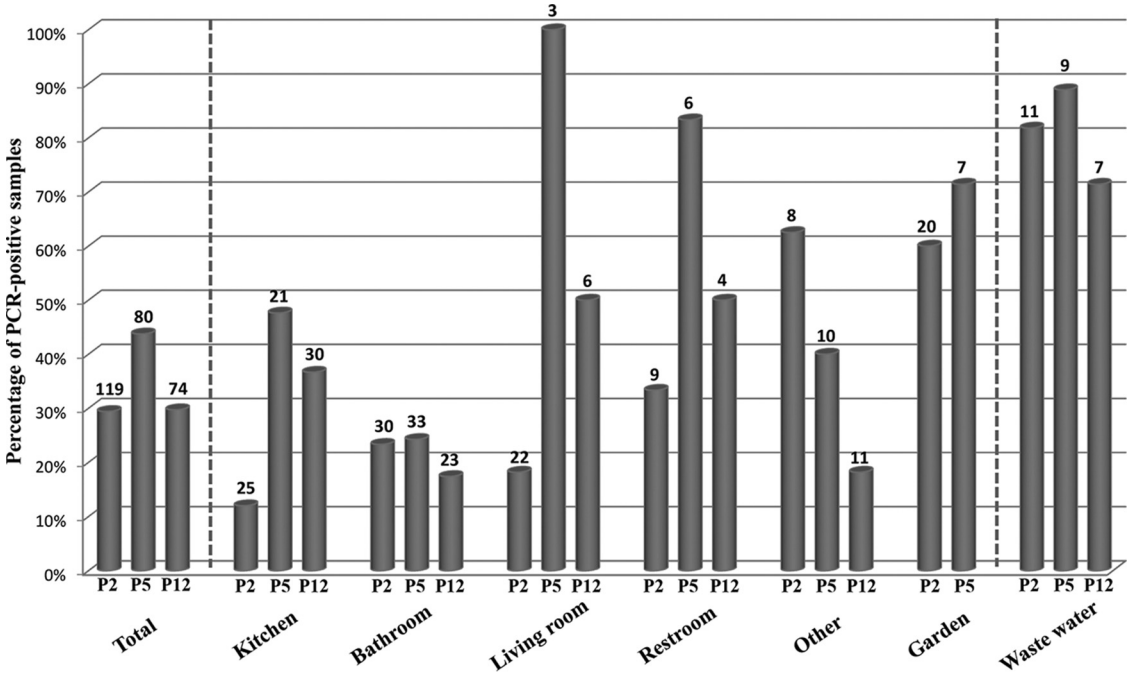

FIG 1 Samples positive by *Achromobacter*-specific PCR: total, per location, and in the wastewaters for the 3 homes studied (those of P2, P5, and P12). The number at the top of each bar represents the sample number.

Cartography of *Achromobacter* in patients' homes. We explored a limited number of CF patients' homes ($n = 3$) (patients P2, P5, and P12) but analyzed a large number of sites per home to accurately delineate *Achromobacter* reservoirs. Positive *Achromobacter*-specific PCR was used for mapping *Achromobacter* habitats. The majority of PCR-positive samples came from indoors (82.5%), mostly from wet sites (78%). The majority of positive samples came from the kitchen for P5 and P12 (28.5% and 50%, respectively) and from the garden for P2 (35%) (Fig. 1). Twenty percent of all the PCR-positive samples came from the bathroom. Wastewater drainage systems were particularly contaminated, and 100% of 11 water samples from siphons were positive by *Achromobacter*-specific PCR (Fig. 1). *Achromobacter* was also highly prevalent in soils and plants samples, at 75% of positive samples.

Considering *Achromobacter* strains cultivated on the selective medium, the origin of the isolates was roughly similar to that highlighted by PCR mapping, and most strains came from the indoor environment, mainly from the bathroom in moist sites, including the bath mat, toothbrush glass, water evacuation of household appliances, siphons (washbasin, dish sink, or shower), and water from the toilet bowl (Table 1). *Achromobacter* strains were also detected in the washing machine drain in two houses. Outdoors, *Achromobacter* grew from three soil samples and one rhizosphere (Table 1).

In our study, water-containing evacuation systems were contaminated by *Achromobacter* spp. but also by several other known CF opportunistic pathogens (17–20), including *Stenotrophomonas maltophilia*, *Bordetella*, *Ochrobactrum*, and *Agrobacterium*/*Rhizobium*. As shown in previous studies, wastewater drains are the primary habitat of *P. aeruginosa* (10–13); we confirm that siphons and other similar draining systems host a large number of opportunistic pathogens. These devices are more and more frequently described as reservoirs for pathogens and multidrug-resistant bacteria in both hospitals and domestic environments (21–23). Moreover, aerosolization and cross-transmission of pathogens from siphons have been demonstrated in recent carefully designed experimental studies (24, 25). Our results reinforced recommendations to CF patients for home cleaning that should be targeted to the bathroom, kitchen, and other wet sites, with particular emphasis on wastewater drains, including hidden drains in domestic appliances. We proposed regular biocidal decontamination of siphons of

water points of use (daily for the more exposing devices or weekly) and annual change of wastewater drains to reduce the global exposure to opportunistic pathogens, including multidrug-resistant bacteria.

High *Achromobacter* diversity in CF patients' homes. *Achromobacter* genotyping performed by multilocus sequence typing (MLST) allowed the accurate identification of species and the description of the sequence type (ST) diversity in CF domestic environments but also the comparison of results to international data. The 21 *Achromobacter* sp. isolates from patient homes belonged to 7 species and 15 STs (Table 1). Only 3 STs exactly matched available STs in the PubMLST database. Therefore, we found in 3 CF patient's homes 12 new genotypes within the genus *Achromobacter*.

Achromobacter mucicolens was the major species identified in this study (8 isolates from 5 samples) and the only one identified in every home. *A. mucicolens* was isolated from various types of samples, e.g., flowerpot, siphons, and toothbrush glass. Five STs were identified among the 8 *A. mucicolens* strains, and strains of 2 STs differing by 2 alleles were coisolated in a soil sample (P5-74) (Table 1).

Five *A. xylosoxidans* isolates were identified in 4 samples in only one home (P5): the first-flush water from the sink faucet in bathroom, the cleaning bucket, the floor cloth, and residual water in the reservoir of the tumble dryer (Table 1). *A. xylosoxidans* ST175 was identified in the 4 samples and associated with *A. xylosoxidans* ST169 in the floor cloth water sample; the 7 alleles of the MLST scheme differed between these two STs. ST175 was identified in the CF respiratory tract of another patient of the Montpellier CF center in 2014 and has been described worldwide from CF and non-CF samples (https://pubmlst.org). ST169 was described only for blood of a non-CF patient with a long-term line for dialysis in United Kingdom.

Other isolates belonged to *Achromobacter aegrifaciens* (2 samples in 2 houses, 3 isolates of 2 STs, including ST143, described for environmental samples in Belgium), *Achromobacter spanius* (2 isolates of different STs in 2 samples from 2 houses), *Achromobacter marplatensis*, *Achromobacter animicus*, and *Achromobacter* sp. genogroup 9 (one isolate each) (Table 1). Of note, the species *A. mucicolens* and *A. animicus* were until then isolated from human samples only. Most species identified from the domestic environment in our study were considered minor CF-associated species in several studies (8, 26–28). For example, *A. mucicolens*, a species described in 2013 on the basis of five human clinical isolates, including one from CF sputum (29), and currently only scarcely reported for CF, was the major species identified in domestic environments in this study. In contrast, *A. xylosoxidans*, the major species identified in CF patients, and chronically colonizing 80% of the CF patients in our center (7), was recovered from only one house.

We observed a high within-species genotype diversity among and within homes, and within samples, as well as one unknown species (*Achromobacter* genogroup 9) and numerous as-yet-unreported genotypes, highlighting a still unrecognized diversity of members of the genus *Achromobacter*.

Antimicrobial susceptibility profile of domestic *Achromobacter* isolates. Isolates were fully susceptible to penicillins, ceftazidime, carbapenems, colistin, levofloxacin, ciprofloxacin, and co-trimoxazole but resistant to cefoxitine, cefpodoxime, aztreonam, and fosfomycin (Table 2). *A. xylosoxidans* and *A. aegrifaciens* showed higher resistance to aminoglycosides and tetracycline than other species (Table 2). Isolates investigated in this study thus exhibited patterns similar to those observed for innately resistant *A. xylosoxidans*, i.e., resistance to many antibiotics, including cephalosporins (except ceftazidime) and aztreonam, with no additional acquired resistances. Only rare studies described the antimicrobial susceptibility profiles of nonhuman *Achromobacter* isolates; they reported resistance to aminoglycosides in *Achromobacter* sp. strains (unidentified species) recovered from non-CF domestic environments in Japan (14) and ciprofloxacin- and/or imipenem-resistant *A. xylosoxidans* strains from non-CF domestic environments in France (100% and 10% of 9 isolates, respectively) (15).

TABLE 2 Antimicrobial susceptibility results for *Achromobacter* strains isolated from domestic environment samples and from the respiratory tracts of the 3 CF patients[a]

Strain	Sample ID or patient	ST	Result (%) for antibiotic tested at indicated cutoff value (in mm)											
			AMX, 19	AMC, 19	PIP, 18–21	TPZ, 18–21	ATM, 21–24	TIC, 15–20	TCC, 15–20	FOX, 15–19	CTX, 15–23	CAZ, 15–18	FEP, 15–18	CF, 16–23
Environmental														
A. animicus	P2-97	360	S	S	S	S	**R**	S	S	**R**	**R**	S	S	**R**
A. mucicolens	P12-61 (1)	349	S	S	S	S	**R**	S	S	**R**	S	S	S	S
	P12-61 (2)	349	S	S	S	S	**R**	S	S	**R**	**R**	S	S	S
	P12-61 (3)	349	S	S	S	S	**R**	S	S	**R**	S	S	S	S
	P2-106	353	S	S	S	S	**R**	S	S	**R**	**R**	S	S	S
	P12-49	359	S	S	S	S	**R**	S	S	**R**	**R**	S	S	**R**
	P5-56	355	S	S	S	S	**R**	S	S	**R**	**R**	S	S	S
	P5-74 (1)	357	S	S	S	S	**R**	S	S	**R**	S	S	S	S
	P5-74 (2)	358	S	S	S	S	**R**	S	S	**R**	S	S	S	S
A. aegrifaciens	P2-20 (1)	350	S	S	S	S	**R**	S	S	**R**	**R**	S	S	S
	P2-20 (2)	350	S	S	S	S	**R**	S	S	**R**	**R**	S	S	S
	P12-26	143	S	S	S	S	**R**	S	S	**R**	**R**	S	S	S
A. spanius	P2-101 (1)	352	S	S	S	S	**R**	S	S	**R**	**R**	S	S	S
	P5-58	356	S	S	S	S	**R**	S	S	**R**	**R**	S	S	S
A. marplatensis	P5-11	354	S	S	S	S	**R**	S	S	**R**	S	S	S	S
Achromobacter sp. gen. 9	P2-101 (2)	351	S	S	S	S	**R**	S	S	**R**	**R**	S	S	S
A. xylosoxidans	P5-71	175	S	S	S	S	**R**	S	S	**R**	**R**	S	**R**	**R**
	P5-64	175	S	S	S	S	**R**	S	S	**R**	**R**	S	S	**R**
	P5-72	175	S	S	S	S	**R**	S	S	**R**	**R**	S	**R**	**R**
	P5-50	175	S	S	S	S	**R**	S	S	**R**	**R**	S	S	**R**
	P5-71	169	S	S	S	S	**R**	S	S	**R**	S	S	S	S
Patient														
A. xylosoxidans	Patient 2	328	S (60)	S (67)	S (100)	S (100)	**R** (100)	S (81)	S (89)	**R** (100)	**R** (100)	**R** (63)	**R** (100)	**R** (100)
	Patient 5	27	**R** (100)	**R** (100)	**R** (100)	**R** (100)	**R** (100)	**R** (100)	**R** (100)	**R** (100)	**R** (100)	**R** (93)	**R** (100)	**R** (100)
	Patient 12	327	S (75)	**R** (62)	S (100)	S (100)	**R** (100)	S (100)	S (100)	**R** (100)	**R** (100)	**R** (50)	**R** (100)	**R** (100)

[a]Sample identification (ID) is composed of the patient number followed by the sample number. S, susceptible; R (in bold), resistant and intermediate. AMX, amoxicillin; AMC, amoxicillin-clavulanic acid; PIP, piperacillin; TPZ, piperacillin-tazobactam; ATM, aztreonam; TIC, ticarcillin; TCC, ticarcillin-clavulanic acid; FOX, cefoxitin; CTX, cefotaxime; CAZ, ceftazidime; FEP, cefepime; CF, cephalothin; CPD, cefpodoxime; IMP, imipenem; MEM, meropenem; GM, gentamicin; TM, tobramycin; NET, netilmicin; AKN, amikacin; FSF, fosfomycin; C, chloramphenicol; TET, tetracycline; CS, colistin; NA, nalidixic acid; OFX, ofloxacin; CIP, ciprofloxacin; LEV, levofloxacin; SXT, co-trimoxazole. Cutoff values were chosen as described by Dupont et al. (35); they consist of the susceptibility cutoff point edited for *Acinetobacter* by the Antibiogram Committee of the French Society for Microbiology in 2016 or, when not available, those edited for *Enterobacteriaceae*. For the 3 patients, the antibiogram result is a percentage of resistance or susceptibility obtained for the majority of the 27 colonies (P2), 15 colonies (P5), and 8 colonies (P12) isolated from the same sputum sample (35).

No link found between house and airways isolates. Patients P2, P5, and P12 were chronically colonized by *A. xylosoxidans* ST328, ST27, and ST327, respectively. No *A. xylosoxidans* isolates were found in the domestic environments of patients P2 and P12. The 5 *A. xylosoxidans* strains isolated in the home of patient P5 belonged to ST175 or ST169. These STs, respectively, differed by 4 and 7 alleles out of the 7 alleles of the MLST scheme from ST27, the ST of the strains recovered from the patient airways. Compared to ST27, each distinct allele differed by 10 to 31 single nucleotide polymorphisms (SNPs) for ST169 and by 2 to 5 SNPs for ST175.

The results suggested either the absence of an environmental reservoir for the strain colonizing the patients or the failure to detect an existing reservoir. Despite the intensive sampling performed, the presence of an undetected reservoir could not be totally ruled out even if a massive environmental reservoir is unlikely. Moreover, the absence of *Achromobacter* on patient personal items such as toothbrush glasses (i) suggested a low rate of transmission from patient to environment, (ii) reinforced the hypothesis of the absence of an environmental reservoir, and (iii) disproved the hypothesis that a domestic reservoir could be involved in the persistence of airway colonization by frequent reseeding. The correlated hypothesis that patients recontaminate their environment could also be rejected. These results suggested that after initial colonization from an environmental source and the subsequent persistence probably associated with specialization to the CF airways, the survival of CF airway-adapted *A. xylosoxidans* strains in the environment could be impaired. This hypothesis is supported by results of previous studies on other CF pathogens. Environmental investigations in the homes of CF patients who were chronically colonized by *Burkholderia cepacia*

TABLE 2 (Continued)

CPD, 21	IPM, 17–23	ETM, 22–25	MEM, 15–21	GM, 17	TM, 17	NET, 16	AKN, 15–18	FSF, 13–16	C, 17	TET, 12–15	CS, 12	NA, 14–19	OFX, 19–22	CIP, 21	LEV, 18–21	SXT, 13–16
R	S	S	S	S	S	S	S	**R**	S	S	S	S	S	S	S	S
R	S	S	S	S	S	S	S	**R**	S	S	S	S	S	S	S	S
R	S	S	S	S	S	S	S	**R**	S	S	S	S	S	S	S	S
R	S	S	S	S	S	S	S	**R**	S	S	S	S	S	S	S	S
R	S	S	S	S	**R**	S	S	**R**	S	S	S	S	S	S	S	S
R	S	S	S	S	S	S	S	**R**	S	S	S	S	S	S	S	S
R	S	S	S	S	S	S	S	**R**	S	S	S	S	S	S	S	S
R	S	S	S	S	S	S	S	**R**	S	S	S	S	S	S	S	S
R	S	S	S	S	S	S	S	**R**	S	S	S	S	S	S	S	S
R	S	S	S	S	**R**	S	S	**R**	**R**	**R**	S	S	**R**	S	S	S
R	S	S	S	S	**R**	S	S	**R**	**R**	**R**	S	S	S	S	S	S
R	S	S	S	**R**	**R**	**R**	S	**R**	S	S	S	S	S	S	S	S
R	S	S	S	S	S	S	S	**R**	S	S	S	S	**R**	S	S	S
R	S	S	S	S	S	S	S	**R**	S	S	S	S	**R**	S	S	S
R	S	S	S	S	S	S	S	**R**	S	S	S	S	S	S	S	S
R	S	S	S	S	S	S	S	**R**	S	S	S	S	S	S	S	S
R	S	S	S	**R**	**R**	**R**	**R**	**R**	S	**R**	S	**R**	**R**	S	S	S
R	S	S	S	**R**	**R**	**R**	**R**	**R**	S	**R**	S	S	**R**	S	S	S
R	S	S	S	**R**	**R**	S	S	**R**	S	**R**	S	S	**R**	S	S	S
R	S	S	S	**R**	**R**	**R**	**R**	**R**	S	**R**	S	S	S	S	S	S
R	S	S	S	S	S	S	S	**R**	S	S	S	S	S	S	S	S
R (100)	S (92)	**R** (100)	S (100)	**R** (100)	**R** (100)	**R** (100)	**R** (100)	**R** (100)	**R** (85)	**R** (100)	S (89)	**R** (70)	**R** (100)	**R** (96)	**R** (85)	**R** (85)
R (100)	**R** (100)	**R** (100)	**R** (100)	**R** (100)	**R** (100)	**R** (100)	**R** (100)	**R** (100)	**R** (53)	**R** (93)	S (93)	**R** (53)	**R** (100)	**R** (100)	**R** (93)	**R** (53)
R (100)	S (100)	**R** (100)	S (75)	**R** (100)	**R** (100)	**R** (100)	**R** (100)	**R** (100)	S (63)	**R** (100)	S (62)	**R** (75)	**R** (100)	**R** (100)	**R** (100)	S (75)

complex bacteria revealed the presence of diverse *Burkholderia* species but did not find the bacterial genotypes colonizing the patients (30). Analysis of the toothbrushes of CF patients colonized by *P. aeruginosa*, of siblings of these patients, and of volunteers showed no statistical relationship between pulmonary colonization and toothbrush contamination (31). Furthermore, studies showed that *P. aeruginosa* strains adapted to CF airways lose their ability to colonize a new host in a murine model and that hypermutable CF strains lose their survival capacity in tap water (32, 33).

Finally, we showed that environmental strains had wild-type antimicrobial resistance phenotypes, while the 3 patients were colonized by isolates displaying higher resistance toward several antimicrobial agents, including ceftazidime, ertapenem, aminoglycosides, and fluoroquinolones (Table 2), suggesting that no diffusion of transmissible antimicrobial resistance determinants occurred between environmental and clinical *Achromobacter* isolates of different genotype or species within domestic environment.

Conclusion. We describe the diversity and the habitat of *Achromobacter* in the domestic environments of CF patients chronically colonized by *A. xylosoxidans*. Despite not being directly involved in the reseeding of CF patient airways during *Achromobacter* chronic infection, indoor proximal wastewater drains appear to be reservoirs for various emerging pathogens in CF. For this reason, we recommend decontamination of such devices. Habitat delineation of *Achromobacter* needs further studies with both colonized and noncolonized patients to better understand the worldwide increased prevalence of *Achromobacter* colonization in CF patients and its possible links to anthropogenic activities, especially to building technology that exposes people at home. This is also true for other water-associated opportunistic pathogens inhabiting technological niches named opportunistic premise plumbing pathogens, such as *P. aeruginosa*, *Legionella pneumophila*, and nontuberculous mycobacteria (34). In developed countries, the emergence of these pathogens leads to increased infectious risk for susceptible patients. This emerging hazard gradually replaces the orofecal risk occurring under low-hygiene conditions and needs particular attention for the development of new indicators and detection methods.

MATERIALS AND METHODS

CF patient home selection. Three patients attending the CF center of the Montpellier University Hospital, in the south of France, who were included in two previous studies on *Achromobacter* chronic colonization, were selected for this environmental study, and their designations were identical in those 2 previous studies and in this one: P2, P5, and P12 (7, 35). The patients were 11, 17, and 19 years old and were chronically colonized by *A. xylosoxidans*, with colonization durations of 5, 10, and 9 years, respectively, at the time of the study. They were all also chronically colonized by *Staphylococcus aureus* and sporadically colonized by *P. aeruginosa*.

The study was approved by the Institutional Review Board (Interface Recherche Bioéthique [IRB]) of the Nîmes University Hospital under IRB number 15/07.05. Written informed consent was obtained from all patients or their parents after explanation of the study.

House and apartment description. The patients live in two houses (P2 and P5) and one apartment (P12) located in 3 different cities of the same region (Occitanie, in the south of France). The house of P2 hosted 4 inhabitants and one cat, the house of P5 hosted up to 8 persons and cats, and the apartment of P12 hosted 3 inhabitants and caged birds. The homes of P2, P5, and P12, respectively, included 8, 10, and 7 rooms and had separated toilets and bathrooms. The home of P5 had a water softener connected to the water supply. The two houses included gardens and swimming pools, either permanent or nonpermanent.

Sampling and bacterial cultures. From 74 to 119 surface and water samples were collected in every home (see the supplemental material). Wet and dry surfaces were, respectively, sampled with dry swabs (Copan) and sponge swabs (Puritan). All swabs were then discharged into 2 ml of Trypticase soya (TS) broth (Becton Dickinson [BD]), and 100 μl of the broth was then inoculated onto TS agar and 500 μl onto an *Achromobacter*-selective agar medium adapted from that of Amoureux et al. (15) consisting of MacConkey agar (MCK; BD) supplemented with 5 g/liter of xylose, 10 mg/liter of aztreonam, 5 mg/liter of amphotericin B, and 5 mg/liter of vancomycin (Bio-Rad). Water samples (250 ml) were collected in bottles supplemented with 20% thiosulfate (Gosselin) and filtered through 0.45-μm membranes (Sartorius). Bacteria retained on membranes were resuspended in 5 ml of filtered tap water by scraping the membrane with an inoculating loop and vortexing for 2 min. Finally, 100 μl was inoculated onto TS agar plates and 500 μl onto *Achromobacter*-selective agar plates. Fifty and 100 μl of other collected liquid samples siphon water, dishwasher evacuation, liquid soap, etc. (see the supplemental material) were directly plated on TS agar, and 300 μl was plated on selective agar. All the seeded agar plates were then incubated at 30°C for 72 h, a temperature allowing the growth of mesophilic bacteria whether of environmental or human clinical origin. For a positive control, we checked that *A. xylosoxidans* clinical strains colonizing the patients were able to be cultivated after 72 h of incubation at 30°C on both the nonselective and the *Achromobacter*-selective media.

***Achromobacter*-specific PCR.** Proteobacterial 16S rRNA gene sequences available in RDP-II (https://rdp.cme.msu.edu/) were aligned with ClustalW of BioEdit software version 7.1.11. Primers were designed in genetic regions conserved in the *Achromobacter* genus only. Primer specificity was tested *in silico* using the PROBE MATCH function within the RDP-II database.

PCR was performed in 50 μl containing 20 pM each primer, 0.2 mM each deoxynucleoside triphosphate (dNTP), 2.5 mM $MgCl_2$, and 0.5 U of *Taq* polymerase (Promega) in the appropriate buffer and 1 μl of DNA extract obtained from a TS-cultivable community. The conditions for amplification were initial denaturation at 95°C for 3 min followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 60°C for 45 s, and extension at 72°C for 30 s, with a final extension step for 10 min at 72°C. Amplification product migration was performed in a 1.5% agarose gel in 0.5× Tris-borate-EDTA buffer containing 500 μg/ml of ethidium bromide, and gels were visualized under UV transillumination. *A. xylosoxidans* clinical strains colonizing the patients were used as positive controls.

***Achromobacter* detection and characterization.** Cultivable communities obtained on TS agar were harvested and homogenized in 1 ml of physiological saline; 100 μl of the suspension was frozen at −80°C in TS broth supplemented with 15% glycerol, and 5 μl was homogenized in 100 μl of DNA-free sterile water for DNA extraction by thermal shock. Crude extracts were subjected to *Achromobacter*-specific PCR as described below. Communities obtained on the specific agar plates were stored at 4°C until the results of the specific PCR were available. In the case of a positive PCR result, the corresponding selective agar plates were screened for *Achromobacter* cultivation; one colony per morphotype (mainly pink and beige) was isolated and identified by matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) using a Microflex LT spectrometer and the MALDI Biotyper database (Bruker Daltonics).

Isolates belonging to the genus *Achromobacter* were subjected to multilocus sequence typing (MLST) as previously described (36), and sequences were analyzed using the *Achromobacter* PubMLST database available at https://pubmlst.org/achromobacter/. The results obtained for environmental isolates were deposited in the *Achromobacter* PubMLST database.

Antimicrobial susceptibility testing was performed on *Achromobacter* species strains using disk diffusion assay as previously described, and results were interpreted according to the breakpoints edited by the Antibiogram Committee of the French Society for Microbiology (http://www.sfm-microbiologie.org/) (CA-SFM 2016) for *Acinetobacter* or, when not available, according to those for *Enterobacteriaceae* (35).

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/AEM.01739-18.

SUPPLEMENTAL FILE 1, PDF file, 0.1 MB.

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